

THESIS

STOCKHOLM

1955

Studies on
BENIGN PROTEINURIA

WITH SPECIAL REFERENCE TO
THE RENAL LYMPHATIC SYSTEM

BY
ERIK LÖWGREN

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TO MY PARENTS

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Introduction

In connection with the clinical investigation of a case of chyluria, published in 1953, my interest was drawn to the renal lymphatic system. On a closer study of the literature on chyluria some features characteristic of this phenomenon seemed to remind me of the so-called "orthostatic albuminuria". Even the most modern text-books on renal topics had still no other explanation as to the mechanism of this proteinuria, however, than hypotheses, incompatible with simple clinical facts. Nor was due consideration taken to the renal lymphatics in the discussion of this renal disorder. A closer investigation of this proteinuria therefore seemed well justified.

Preliminary reports on this work have been delivered (LÖWGREN 1952), and papers have been read before The Swedish Association for Internal Medicine and a Scandinavian Meeting on Renal Topics (May 1954) and before the 24th Scandinavian Congress of Internal Medicine (Sept 1954).



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Nomenclature and definition

As early as the 19th century, after Bright had published his works on the connection between proteinuria and renal disease, it was evident to many investigators that protein may well appear in the urine of healthy kidneys. A copious literature presenting many different aspects on this urinary protein has since then appeared, and the nomenclature is very varying. Most designations, however, are of mere historical interest, and in fact many of them are synonyms (*e.g.* benign, harmless, physiological; transitory, intermittent, cyclic; adolescent, juvenile; etc.). Denominations as postural (STIRLING 1887), orthostatic (TEISSIER 1899), or orthotic (v. STEJSKAL 1908) stress the influence of the stature of the body, and lordotic (JEHLE 1908) the importance of the lordosis of the spinal column. The subject of nomenclature has been further complicated by designations as non-lordotic-orthostatic and non-orthostatic-benign (PARKIN 1951). It is, however, obvious that this proteinuria preferably appears in young people, but it may occur in old individuals, too (BULL & JONES 1948).

Although the diagnostic methods for deciding the state of the renal health have improved during the past decades, the imperative criterion of this proteinuria has always been the absence of signs of renal disease.

The prognosis is also in modern literature regarded as excellent (FOX 1921, SALVESEN 1948, FISHBERG 1954) and it must indeed be seen in the light of *e.g.* HAMILL & BLACKFAN'S (1911) proving the existence of such proteinuria in nearly 90 per cent of children, and the data of BULL (1948), who could induce it in three-fourths of young people.

It is difficult to propose a uniform designation. But rightly the term "albuminuria" is more and more abandoned and replaced by the more adequate "proteinuria". In my opinion this proteinuria should probably be assigned to one and the same renal mechanism, however, even if certain characteristic features, such as the above mentioned, have been more or less pronounced in the materials investigated by the various authors.

By the term "benign proteinuria" is in the following meant that form of proteinuria which appears without provable connection with renal disease. It is especially common in growing young people and a positive correlation to the stature of the body, especially the standing position, and the lordosis of the spine, is obvious.

A survey of the literature, with special reference to earlier theories of the cause of the benign proteinuria

Just as concerning the nomenclature, there are many variations given in the literature as to the theories on the cause of the proteinuria. However, practically all modern authors on the subject assign it to disturbances in the renal blood supply, and especially to a hindrance of the venous return from the kidney.

Older theories, such as mal-nutrition, pre-tuberculous constitution, abnormities of the blood chemistry, irritation of the sympathetic nerve supply of the kidney, etc., are left out in the following. Congenital changes without a closer definition have also been suggested even in modern literature (RYTAND 1937, ADDIS 1948, PARKIN 1951, KING & GRONBECK 1952). The conception of some "Dysproteinämie" held by WUHRMANN & WUNDERLY (1950) is not shared by other authors (see FISHBERG 1954).

The influence of the body position (orthostatic = standing upright) has been mentioned above. Observations of well defined benign proteinuria in the supine position have also been reported (ANDERSSON 1943, ADDIS 1948).

In 1913 JEHLÉ indicated the importance of the lordosis and postulated a venous stasis due to the lordotic columna. If related to a stretching or a bending of the veins he left out of discussion, as well as the very place of that venous disturbance. The great importance of the lordosis has since been confirmed by many investigators, but it does not seem to be solely decisive (NOWAK 1934, KING & GRONBECK 1952 and others).

On the basis of JEHLÉ's lordotic theory, SONNE (1920) put forth his theory of stasis of the left renal vein, with a striking explanation of the compression of this vessel by the lordosis. As early as 1908 NOVAK presented investigations on the mechanic effect of the lordotic spine on the renal vessels, and he could not observe any changes of the diametres of those vessels. Other investigators arrived at another possible mechanism for a supposed pinching of that vessel, *i.e.* by means of adjacent viscera such as the mesenteric vessels (KELLING 1919, RIESER & RIESER 1922, KRISTENSSON 1926). Benign proteinuria has however been observed to come from the right kidney, too (EKEHORN 1931, BULL 1948).

BULL therefore assumed the existence of a more central venous stasis mechanism. On the basis of extensive studies of the literature and own investigations he presented a theory of a compression of the inferior vena cava in 1948. This was thought to be compressed against the spine by the posterior surface of a rotated liver, but only in the lordotic position. In a final footnote of his thesis he said—on the contrary—that the influence on the caval vein might be located at the diaphragmatic opening (moreover this theory was earlier proposed by EPPINGER 1912).

BULL's theory does not seem to explain the proteinuria occurring without the lordosis. The common conception of the role of a venous stasis phenomenon in connection with benign proteinuria is not supported by the investigations by

LAGERLÖF, ELIASCH, WERKÖ & BERGLUND (1951) regarding the venous blood pressure in the standing and the supine position, where an increase in the systemic pressure was found in the horizontal position as compared with the upright.

Other authors have been apt to regard changes in the arterial circulation as the causative factor. First of all the so called orthostatic mechanism may be mentioned, *i.e.* the change of the arterial circulation and the blood pressure that appears in the standing position. As this "orthostatismus" is fairly common in young growing individuals, particularly in those displaying an asthenic build, and the benign proteinuria very prevalent for the same age group, such a connection seems to be near at hand. ERLANGER & HOOKER (1904) demonstrated a correlation between proteinuria and changes in the pulse pressure. v. STEJSKAL (1908) assigned a decisive importance to the vasomotoric reaction in the standing position. In Sweden BJURE & LAURELL (1927) have emphasized the possibility of explaining the proteinuria by the orthostatic arterial mechanism and the accompanying changes in the distribution of the abdominal blood. Investigations of larger materials have not proved any connection, however, between the arterial blood pressure or pulse pressure and the proteinuria (BULL & JONES 1948). FISHBERG (1954) also doubts the connection between this proteinuria and alterations in the general arterial circulation.

An intra-renal vasoconstriction as the causative mechanism has been postulated by STARR (1926) based on experimental investigations on animals and on humans, and by WHITE & ROLF (1948) based on investigations on healthy people. But these results do not agree with the common appearance of high grade proteinuria in quiet standing diminishing or even disappearing in motion.

The common finding of proteinuria in animals, by ligating the renal artery or the vein has been put forth as a support of the "arterial" and the "venous" theories.

Now, if the opinions of the causing factor in the benign proteinuria vary, there is a greater unanimity as to the place where the protein is believed to enter the urine. In the works where this question is more thoroughly discussed, the authors generally regard the glomerulus as the locus for the passage of the protein from the plasma to the urine. This theory was held already by HEIDENHAIN (1883) and SENATOR (1902), and is so by modern authors (EKEHORN 1931, MEDES & NEEMES 1935, BING 1936, DRINKER 1942, IVERSEN, BJERING & BING 1946, SALVESEN 1948, WHITE & ROLF 1948, ALLEN 1951, SMITH 1951, FISHBERG 1954 and others). Even though the mechanism is sometimes spoken of more commonly as an increased permeability in the kidney membranes, or a state of "leaky kidney" (*e.g.* WALLIS 1920) it is clear that this leakage is supposed to take place in the glomerulus. The cause of the proteinuria *per se* is then meant to be explained by an increased permeability in the glomerular filter for protein. The very mechanism for that increased permeability, however, is not satisfactorily explained, but the theory agrees with the modern conception of the mechanism of the proteinuria in the different forms of renal diseases.

A divergent opinion of the benign proteinuria was held by ADDIS (1948) who explained it by a "definite degenerative renal lesion" in the tubulus, however of a short duration. The rôle of the tubulus in the renal handling of the protein on the whole has, as is well known, been especially defended by him. On the basis of a long-time observations of a small group of proteinurics ADDIS also put forth a theory of a apparently benign form of "prerenal proteinuria" (ADDIS 1945, 1948). However, further investigations in this line did not seem to verify his hypothesis (ADDIS, BARRETT, POO & UREEN 1951).

Several facts taken from the literature do not support the conception of the glomerular rôle in benign proteinuria. A tendency of cylindruria is missing as is also hematuria (NAERAA 1936) and even high protein values without casts have been observed (WOLMAN 1945). JEHL (1913) determined an appearance time for protein in the bladder urine of 20 -30 seconds in one case, which contradicts a glomerular change. Other functions of the glomerulus, such as the filtration, are lower in the standing than in the supine position (RYTAND 1937, BULL 1948, SMITH 1951). MEDES & NEEMES (1935) could prove a lower clearance of creatinine in the standing lordotic position, associated with a simultaneous high protein output.

Against the glomerular filtration of the protein and in some degree also against the possible rôle of the tubulus argues the qualitative nature of the urinary protein. For a long time it has been an evident fact that globulins are excreted along with albumin (HAMILL & BLACKFAN 1911, WALLIS 1920 and others). By more modern methods for the analysis of the proteins it has been demonstrated that for instance electrophoretically the urinary proteins do not differ from those of serum (SCHMIDT 1953). It has been demonstrated by RIGAS & HELLER (1951) that the globulins do not diverge as far as their sedimentation velocity in the ultracentrifuge is concerned. These facts cannot simply be fitted into current theories of the glomerular permeability for plasma proteins, even if COHN's conception of the plasma proteins (sausage-formed bodies of about the same diameter) is considered.

Whatever be the causative circulatory mechanism in the kidney, it must imply a rather essential alteration in the functions of the renal cells or membranes, to permit the passage of those proteins. Such supposed alterations are contradicted to some degree by the fact, that these kidneys do not show any tendency to later inflammatory or degenerative parenchymal diseases. The few cases which have been submitted to a post mortem study have not presented any histological alterations in the nephron (RYTAND 1937, ADDIS 1948, SALVESEN 1948) (which of course does not give a reliable evidence of the functions of those nephrons *in vivo*).

From a practical clinical point of view the frequently observed intermittency of the proteinuria (variations from day to day or within a day) does not speak in favour of a disturbance of the blood circulation as the releasing mechanism. An interesting observation of seasonal variations, which can hardly be put in connection with any such mechanism either, was made by MURPHY (1944).

A hypothesis, completely differing from the above, was presented by QUINCKE (1912). Not until I had published my first communication in 1952 did I find his report. Under the title "Lymphurie?" QUINCKE put forth a theory of the admixture of lymph as the plausible explanation of the benign proteinuria, based on the intimate anatomical connection between the renal lymphatics and the nephron and the lower urine passages. Moreover QUINCKE pretended to have put forth his theory as early as 1876. I have not, however, been able to find it in the text-book he mentioned (ZIEMSEN'S Handbuch der speziellen Pathologie und Therapie).

On the basis of the introductory facts, I have arrived at the same reasoning myself. As QUINCKE did not further support his theory by any investigations, nor definitely mentioned the possible place of that admixture of lymph-protein, and above all as he has not been referred to in modern literature, I have found it well justified to carry on my investigations.

To sum up from the above review of the literature, it appears that partly contradictory theories have been put forth as far as alterations in the blood circulation are concerned. Neither can one of these be accepted as the mechanism of the proteinuria *per se*, i.e. the passage of the protein from blood to urine. The very place of this passage (a very important question in my opinion) is assigned to the glomerulus by practically all authors. Data from the literature on the benign proteinuria do not seem to make such an opinion acceptable. And there are some data indicating that the place of the protein admixture should be looked for distally of the nephron. The source of the protein may in that case be the renal lymphatic system, which will be reviewed in the following chapter.

Review of the renal lymphatic system

The headlines of the topographical anatomy were given as early as 1787 by MASCAGNI, who has also been duly honoured with fine reproductions in the monography by NARATH (1951). Further investigations stressing the intimate connection with the lymphatics of the other abdominal viscera and the genitals were made by BARTELS (1909) and HASUMI (1939). KUMITA (1909) presented works on the lymphatics of the capsules of the kidney. Most of the older works, however, were carried out by means of various injection procedures, often leading to results difficult to interpret. For further references with different historical aspects, see ABESHOUSE (1934) and NARATH (1951).

In the modern literature there are now several investigations available, which prove the existence of a richly developed lymphatic system in the renal parenchyma as well as in the fibrous and the adipose capsule and in the pelvis and the lower urinary passages in man (HELMKE 1938, KAISERLING 1940 & 1942, FRESSEN 1943, PEIRCE 1944, RAWSON 1949, BABICS 1951, NARATH 1951, GIRGENSOHN 1952 among others).

Briefly this lymphatic system can be summed up thus: in the perirenal tissue with its adipose capsule as well as in the fibrous capsule there is an extended network of lymphatics, communicating with one another and with the surrounding viscera. Further there are rich communications with the lymphatics of the renal parenchyma, where net-works of lymphatic capillaries surround the glomerulus (Bowman's capsule) and the tubular system. Lymph capillaries also lie adjacent to the arterioli of the glomerulus. Connecting lymphatics from the renal cortex and the medulla confluence at the cortico-medullary border into larger trunks, into which also vessels from the pyramid enter. Running alongside with the blood vessels the larger lymphatics take their course towards the sinus renalis (Henle)¹ Lymphatic trunks from the pelvis and the proximal ureter also enter this sinus. The total drainage of these lymphatics then follows by means of several (usually 4—8) larger trunks, which leave the kidney through the hilum, running close to the artery and the vein, taking their course medially to lymphatic glands near the aorta and the lower caval vein. Communicating with vessels from adjacent viscera and the genitals, the lymph takes its further course *via* the truncus intestinalis through the diaphragm on its further way upwards in the thorax (ductus thoracicus).

A richly developed lymphatic network is to be found particularly around the glomerulus, the tubuli contorti and the loop of Henle (KAISERLING) and in the sinus renalis in the region of the fornix of the calyx (HELMKE; NARATH). The lymphatics are built up by an endothelium and sparse connective tissue, as generally in the lymphatic system. Muscles do not seem to be part of the renal lymphatics. Not until the larger trunks pass the limit of the parenchyma do valves appear. NARATH also states that lymphatic capillaries are included between the loops in the glomerulus, a fact refuted by others.

In his studies, KAISERLING used the "physiologic" method of making the lymphatic vessels visible by ligating the larger trunks outside the kidney. His experiments on rabbits, together with SOOSTMEYER (1939) may be designed as classic. They obtained thereby among other results an increase of the kidney volume to nearly double the size in 15—20 minutes after the ligature was applied. KAISERLING later (1940 & 1942) used this technique for further studies of the anatomy of the renal lymphatics and their rôle in various patho-physiologic connections. He illustrated his works with very good reproductions and he seems to have emphasized the rôle of the renal lymphatic system in normal and pathological physiology more than anyone else. Under the same simple experimental conditions he has, for instance, obtained a tubular-nephrotic syndrome.

The histological connection between the renal lymphatic system and the lipoid-nephrosis and the amyloidosis in man was also stressed by HELMKE (1938) and

¹ Sinus renalis Henle designs the anatomic space within the kidney, which is surrounded laterally by the inner surface of the parenchyma, and medially by the intrarenal portion of the renal pelvis with its calyces. It is filled out with fatty tissue with discrete connective fibers, imbedding blood vessels and lymphatics.

FRESEN (1943). FREY (1949) reported on a case of lipoid-nephrosis in connection with a wide-spread stasis of the abdominal lymphatics.

BABICS (1951) reported investigations particularly on the lymphatics of the pelvis and the ureter by means of ureteral ligature in animals (specimen not mentioned), referring especially to the hydronephrosis and the peri-renal infections. His report is also completed by several histological reproductions.

The importance of the lymphatic system has long been known as far as the suppurative infections and the tuberculosis of the kidney and the pelvis are concerned. Modern investigators seem to have paid less attention to its existence in their works on the various forms of the non-bacterial nephritis, the nephrotic syndrome and so on, with the above exceptions.

As to the renal pelvis there is especially one anatomically well defined region that seems to be connected with some well-known medical conditions, namely the fornix of the calyx. NARATH assigns a great importance to this region, "in which absorptive processes seem to be accomplished more than anywhere else in the transportation system of the urinary tract" (NARATH 1951). There exists a richly developed lymphatic net-work in the sinus renalis close to the fornix. In the very same region the so-called pyelolymphatic refluxes, well-known in the roentgenologic literature, take place in intravenous or retrograde pyelography (ABESHOUSE 1934, OLSSON 1948, NARATH 1951). As to this phenomenon NARATH maintains the possibility of mere reabsorption, *i.e.* without mechanical rupture of the endothelium, and proposes the term "fornical absorbtion", but prefers the broader term "pyelousinous transflow", as the exact knowledge of the process is still a matter of dispute. Mechanical ruptures of course occur in several cases where the reflux is indisputable and of a high degree. By means of such refluxes of the contrast medium the outlines of lymphatic vessels have been made visible in several materials (*e.g.* WOOD 1929, CAMPBELL & SEIDLER 1937, OLSSON 1948, NARATH 1951, LöwGREN 1953). As a mere curiosity may be mentioned a report by SHIMIZU (1937), who by the retrograde injection of thorotrast in the renal pelvis was able to distinguish the lymphatic route as far as the level of the second thoracic vertebra in man.

The importance of the fornix of the calyx in the renal pelvis in patho-physiological relations was even stressed by HELMKE (1938) and GIRGENSOHN (1952). By means of ureteral ligature in animal experiments the latter investigator obtained histological changes in the pelvic endothelium in that region, and these changes were observed considerably earlier in pregnant animals.

From the clinical point of view the same area is of interest, as the passage of chyle in chyluria takes place in the same region, in those cases where the discharge occurs in the upper urinary passages.

The reported phenomena, which of course do not agree with quite physiologic conditions, speak in favour of the existence of a *locus minoris resistentiae* in the

fornix of the calyx. Its possible rôle in normal pelvic function has, however, been emphasized by NARATH and GIRGENSOHN.

The literature on chyluria gives instances of the fact that hydrostatic and mechanical factors may influence the flow of the abdominal lymph. Changes in the position of the body have been shown to alter the discharge of chyle into the urine (QUINCKE 1876, CHARTERIS 1911, RICHTER 1928, WOOD 1929, LAZARUS & MARKS 1946, among others). Intraabdominal pressure changes (*e.g.* a pregnant uterus) have been pointed out as possibly having a similar effect (SANES & KAHN 1916, RAY & RAO 1939) and even voluntary straining of the abdomen has been shown to have such an influence (RAY & RAO). Chyluria further gives an example of a post-renal proteinuria (*i.e.* protein admixture distally of the nephron) with intermittency, spontaneous ceasing after short or long intervals, excellent prognosis as to the renal parenchyma (where the primary disorder does not decide otherwise) and a prolonged loss of protein without a deleterious effect on the general physical condition or on the plasma proteins.

The varying factors that have been said to have influence on the benign proteinuria, such as the body position, the lordosis of the spine, intra-abdominal pressure alterations (*e.g.* by means of girdles, pneumatic suits, manual pressure, the sinking of the body in water, a pregnant uterus, etc.) will no doubt at first hand affect the fluid system, circulating under the lowest pressure, *i.e.* the lymph. As the kidney, according to KAISERLING, may be regarded as one of the human organs most rich in lymph, and the lymphatic system is especially richly developing in the adolescence, there are certainly good reasons to presume a connection between the lymphatics and the benign proteinuria.

There are no reports to be found in the literature regarding the protein contents of human renal lymph. In chyluria, where of course any part of lymph deriving from the kidney cannot possibly be determined, urinary proteins have been shown to include both albumin and globulin (HERTZ 1907, RAY & RAO 1939, LAZARUS & MARKS 1946).

Experimental investigations on renal lymph have been performed by *e.g.* SCHMIDT & HAYMAN (1929), where the influence of diuretics on the flow of lymph was studied in dogs. KAISERLING & SOOSTMEYER (1939) carried out electrolyte analyses on renal lymph in rabbits. FREY (1949) determined the cholesterol concentration in rabbits. SUGARMAN, FRIEDMANN, BARRETT & ADDIS (1942) studied the distribution, flow, protein and urea content of renal lymph in dogs. The protein concentration in their experiments varied between 0.4 and 4 gram per cent, and rising values were observed parallelly with the decreasing rate of the lymph flow. By studying the distribution of inulin and glucose in dog's lymph, KAPLAN, FRIEDMANN & KRUGER (1943) arrived at the conclusion that renal lymph is made up of blood plasma and re-absorbed tubular fluid. CARLSTEN (1950) proved by means of experiments on cats that the transport of histaminase from the kidney occurs *via* the lymphatics.

The protein contents of human thoracic lymph were determined *in vivo* by BIERMAN *et al* (1953). The concentration varied with and was slightly lower than that of the corresponding plasma.

To sum up from the above accounts on the renal lymphatic system, a working hypothesis based on a post-renal admixture of protein seems well justified, in order to contribute to the comprehension of the mechanism of the benign proteinuria.

PART TWO

OWN INVESTIGATION

Introduction

My first attempts to study this proteinuria in some healthy young people with the aid of ureteral catheters were soon abandoned. Such a method cannot be looked upon as physiologic, especially not when the lower urinary passages are concerned. Animal experiments (on dogs, cats, and rabbits) have given me an insight into the anatomy of the renal lymphatics but have also convinced me of the delicate building of this system and the difficulties in the handling of these threadfine vessels. As no results of experimental investigations on such animals could be justly transferred to two-legged beings, no animal experiments are included in this paper. This is instead based on investigations of some healthy males and females, with the chief stress laid upon the analysis of their urines, and the discussion will mainly deal with the renal mechanism proper for the proteinuria.

Material including case reports

The study embraces nine males (17—22 years of age) and eight females (15—21 years of age). Apart from protenuria those have been subjectively and objectively healthy at the time of my investigations. No signs of renal disease have appeared, with the exception of one case (case KL), as far as urinary sediments, non-protein nitrogen of the blood, and urography are concerned. In most cases the renal capability of concentration and dilution of the urine has been estimated, too. All the cases have been known to have proteinuria of the benign type since a short or a long time, or have been admitted to this hospital because of recent discovery of proteinuria in routine examinations for healthy certificates or the like, still with the exception for case KL. Two cases (UB & LW) have been prescribed bed-rest by their physicians for a period of some weeks one year before my study began, but signs of glomerulo-nephritis or nephrosis are lacking in their case histories. One case (SK) has suffered a benign bacterial cystitis for a short period during pregnancy one year before my investigation.

As to the sediments, several specimens from each individual, including one or more night-urines as well as several specimens during day-times, have been

examined. Pathologic formed elements in the form of casts have never appeared, and erythrocytes above the normal range has been established in case KL only. This girl gives a history of several upper respiratory infections including tonsillitis and otitis, and a passed haemorrhagic glomerulo-nephritis must be looked upon as suspicious from a clinical point of view. At the time of my study a clinical examination of this girl, however, gave a fully normal result with the exception of proteinuria and microscopic haematuria.

No symptoms of genitary inflammations have occurred in the material.

The cases are denominated by the initial letters of their names. Their records are to be found at the department of internal medicine in this hospital, its out-patient department or its department for occupational medicine.

The laboratory procedures mentioned in the case reports have been performed as follows:

Blood pressure (BP): sphygmomanometre, auscultated values in mm Hg (brachial artery).

Non-protein nitrogen in blood (NPN): according to FOLIN & WU (1919), *i.e.* laked blood, in mg per cent.

Specific gravity of the urine (sp.gr.): urometer, with correction made for temperature.

Urinary protein (qualitative method): Heller's nitric acid test by filtering the urine over 25 per cent nitric acid, or by pipetting the acid below the urine. The conception of its sensitiveness is varying in the literature, but a negative "Heller" can safely be said to exclude significant amounts of protein, *i.e.* more than 0.05 per mille (HAMMARSTEN 1947). For the purpose of my study this is satisfactory.

Creatinine in plasma and urine: according to LIEB & ZACHERL (1934).

Urography: see page 28.

By "night urine" (NU) is meant the specimen passed at the awakening in the morning.

The dilution ability of the kidney has been estimated after the drinking of one litre of water, as a rule on an empty stomach.

The sediments in the case reports have been judged from a qualitative point of view only and with special regard to erythrocytes and casts. The usual laboratory method has been centrifugation at about 1,500 rotations PM for 3 minutes.

Notations on protein concentrations are omitted in the case reports, the method employed (Esbach's picric acid test) being too inaccurate. With the exception of case GA (before delivery) the protein concentration has in no case been remarkably high.

Case reports

Males

BL Born 5/3 1933. Military man. Always been healthy. Proteinuria revealed at a routine examination for healthy certificate. Out patient department (OPD): Dec-54. BP 125/70. NPN 28. NU sp.gr. 1.031, protein 0. Day-time urines: protein 0. Sediments normal.

LV Born 1/9 1932. Military man. Always been healthy. Proteinuria at a routine examination in connection with a common cold. OPD: Oct-54. BP 125/85.

- NPN 30. NU sp.gr. 1,033, protein 0. Day-time urine: protein trace. Water-test: conc. 1,033, dilution 1,005. Sediments normal.
- dR Born 10/9 1936. High school student. Always been healthy but benign proteinuria known since some years.
OPD: Nov-54. BP 125/85. NPN 31. NU sp.gr. 1,031, protein 0. Day-time urine: protein +. Sediments normal.
- BD Born 26/12 1935. Goldsmith. Always been healthy. Benign proteinuria known since school.
OPD: Oct-54. BP 135/85. NPN 30. NU sp.gr. 1,027, protein 0. Day-time urine: protein 0. Water test: conc. 1,027, dil. 1,003. Afternoon urines: protein trace. Sediments normal.
- BM Born 16/6 1937. Shop assistant. Always been healthy. Proteinuria revealed at an examination for healthy certificate.
OPD: Oct-54. BP 120/70. NPN 33. Daytime urine: sp gr. 1,027, protein 0. Sediments normal.
- KE Born 16/9 1934. Military man. Benign proteinuria known since school. Arterial hypertension revealed in 1952. Never subjective symptoms.
OPD: Nov-54. BP 165/90. NPN 30. NU sp.gr. 1,029, protein 0. Water-test: conc. 1,033, dil. 1,004. Daytime urine: protein +. Afternoon: protein trace. Sediments normal. X-ray of the heart: normal picture. Physical working capacity (according to WAHLUND 1948): normal, and without decompensatory symptoms as to the heart. Electrocardiograms normal. Phonocardiogram normal.
- AS Born 1/7 1936. Typographer. Always been healthy. Benign proteinuria known since school.
OPD: June-54. BP 130/80. NPN 33. NU protein 0. Daytime urine: protein +. Sediments normal. Dil. 1,003.
- OL Born 4/8 1936. Junior clerk. Always been healthy. Proteinuria revealed at routine examination for healthy certificate.
OPD: June-54. BP 120/80. NPN 32. NU sp.gr. 1,025, protein 0. Dil. 1,002. Daytime urine: protein +. Sediments normal.
- KH Born 20/6 1937. Cook. Earlier healthy but repeated pharyngitis in the summer of 1954. Proteinuria revealed at routine examination for healthy certificate.
OPD: Oct-54. BP 125/75. NPN 28. NU sp.gr. 1,030, protein 0. Dil. 1,002. Daytime urine: protein +. Sediments normal.

Females

- AW Born 9/9 1938. School girl. Always been healthy. Benign proteinuria known since one year. Medical department (record No 2001/53). BP 130/70. NPN 32. NU protein 0. Water test: conc. 1,028, dil. 1,003. Daytime urine: protein +. Sediments normal. Urography normal.
- UB Born 26/2 1937. Waitress. Always been healthy. Healthy certificate refused in Nov-53 because of proteinuria and prescribed bed rest for a month. Later examinations normal till Oct-54, when proteinuria was revealed.
OPD: Oct-54. BP 120/75. NPN 35. NU sp.gr. (lost). Protein 0. Dil. 1,003. Daytime urine: protein +. Sediments normal.
- LW Born 27/5 1937. Lady clerk. Always been healthy but proteinuria revealed at healthy certificate examination in the summer of 1953, and prescribed rest for two months. Later examinations still proteinuria.

- OPD: Sept-54. BP 145/85. NPN 30. NU sp.gr. lost. Protein 0. Dil. 1,003. Day-time urine: protein +. Sediments normal.
- SK Born 17/4 1933. Waitress. Always been healthy except a banal cystitis in pregnancy 1953. Benign proteinuria known since March-54. During the summer this year a tendency to oedema of the legs. Examined in another hospital in August-54, where nothing abnormal except proteinuria was revealed. Urography then normal. OPD: Oct-54. BP 125/75. NPN 29. NU protein 0. Water test: conc. 1,033, dil. 1,004. Day-time urine: protein +. Sediments normal.
- GF Born 11/3 1934. Medical gymnast. Benign proteinuria known since childhood. Her father physician. Repeated pharyngitis in autumn-54. OPD: Nov-54. BP 110/80. NPN 32. NU protein +. Daytime urines: protein +. Sediments normal. Water test: conc. 1,028, dil. 1,002.
- GK Born 26/2 1939. School girl. Always been healthy till the autumn-54, when she revealed oedema of the legs and proteinuria. OPD: Oct-54. BP 145/95. NPN 25. NU protein 0. Water test: conc. 1,029, dil. 1,004. Daytime urine: protein trace. Sediments normal. Oedema gone. Serum protein value normal (8 gram per cent).
- GA Born 7/9 1934. Lady clerk. Always healthy but benign proteinuria known since 1952. During pregnancy a massive proteinuria developed about 3 weeks before the normal delivery (in Jan-54), and oedema and low serum protein values were revealed during her stay at the obstetrical department. Repeated examinations of BP always normal, as repeated NPN-determinations. Repeated sediment examinations never revealed any erythrocytes or casts. Immediately after delivery diminishing protein output, increasing serum protein and disappearance of the oedema. Medical department May-54. BP 115/70. NPN 28. NU protein + (in the magnitude of 1—2 per mille). Water test: conc. 1,028, dil. 1,000. Sediments normal. Catheter urine: 0 bact. Culture neg. Exogenous creatinine clearance (supine): 147 ml/min, standing 109 ml/min. Urography normal.
- KL Born 15/11 1938. School girl. Otitis in 1941. Repeated sore throat attacks in the years 1942—1948. Adenoid ectomy (epipharynx) performed in 1944. Since May-54 back pain, especially in standing position, and tiredness. Proteinuria revealed at school in Oct-54. Med. department Jan-55. BP 125/75. NPN 25. Repeated protein tests on night-urines and in day-time specimens positive (in the magnitude of 0.5—1 per mille) and in practically all sediments erythrocytes above the normal range, but not more than 10 red cells per view field with the usual magnification. Never casts. Catheter urine: 0 bact. Culture neg. Exogenous creatinine clearance (supine) 219 ml/min. Urography normal.

The investigation

The individuals reported on were investigated in the following manner: on the arrival in the out-patient department (after having breakfast as usual at home) the bladder was spontaneously emptied, and the urine kept for analysis. Immediately afterwards the person in question was placed in the following standing position: feet with heels together at a distance of about 1 decimetre from the wall

and legs rotated outwards. Back of the neck and shoulders leaning against the wall and abdomen pushed forward induced a form of standing lordosis. This position was held for 5 minutes. No symptoms of "orthostatismus" in the form of fainting or the like appeared, and the position cannot possibly be looked upon as being remarkably unphysiologic. The standing lordotic position has been shown by many authors to induce a high grade proteinuria (JEHLE 1913, KRISTENSSON 1926, BULL 1948 among others). After standing for five minutes, urine was voided again spontaneously as soon as possible. In some cases this was accomplished in a few minutes, in some, however, not until ten minutes or more had passed after the lordotic position. Only in isolated cases a glass of water was given before the second specimen was voided. In some cases the standing lordosis was induced twice before the second voiding.

My assumption was, that the urinary protein appearing in connection with the lordosis does not differ as far as its quality and the renal mechanism of its passage to the urine are concerned, from the protein that might appear in the urine before lordosis. My purpose was to analyse the two urine specimens with regard to the protein, the sediment, and the creatinine concentration.

The urine was in all cases voided. This of course made the interpretation of the sediment difficult. But as the purpose was to study mainly the possible appearance of erythrocytes and casts, the cells from the outer urethra or the vulva did not interfere.

Blood was taken by venipuncture in the cubital fossa, and for serum analyses the blood was left for spontaneous coagulation. For eventual plasma analyses blood was taken in bottles prepared with heparin.

Urography had been performed in some cases (AW, SK, GA & KL) before my own study. In the other cases it was made in connection with this, *i.e.* one or two weeks before or after my study. The significance of the time relation between the urography and the lordotic study will be further discussed in connection with the results of the roentgenexaminations.

Own laboratory methods

The sediment: the urine was centrifugated immediately after voiding the specimen, in an ordinary laboratory centrifuge at 2,000 RPM for 3—4 minutes. The supernatant fluid was kept for further analyses. The sediment, in most cases macroscopically noticeable, was mixed up again thoroughly in the smallest possible volume of urine, laid up upon a glass, covered by a glass 18 by 18 mm, and investigated with two magnifications (objective 8 resp. 40; ocular 7, Zeiss' microscope). The notations in the following as to formed elements per view field regard the higher magnification. The whole sediment under the covering glass was carefully gone through. The method gives of course no "quantitative" dates, but on the other hand most of the sediment of the specimen in case was examined.

The urinary protein: preliminary estimation was made with the nitric acid test by pipetting the acid under the urine in a small test tube. Protein “+” in tables means a distinctly positive reaction, and “++” means a strongly positive, i.e. massive coagulation of the urine.

Quantitative method: The Biuret-method worked out by HILLER, GREIF & BECKMAN (1948) has been sufficiently sensitive for my purposes. The range of error in the total protein concentration is no greater than ± 5 per cent (CHINARD, LAUSON, EDER, GREIF & HILLER 1951). For further checking of the method as far as different protein concentrations in urines from the same individual are concerned, the following procedure was undertaken: a serum of known protein contents (6.2 gram per cent by Kjeldahl) was diluted in a geometric series with

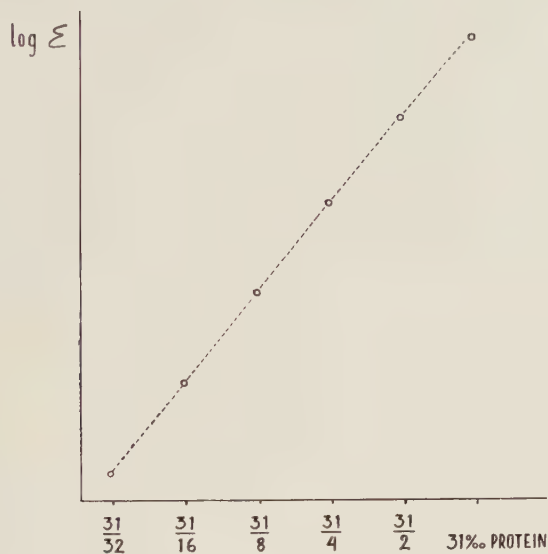


Fig. 1.

a morning urine (protein-free by the nitric acid test) of high specific gravity, and the biuret-method was performed on the various dilutions. All values obtained through this method are the results of concordant duplicates. As can be seen in figure 1 the exactness between one and thirty per mille is satisfactory. In the tables values below 5 per mille are given with one decimal. The protein values in the urines are as a rule given in per mille (i.e. gram per 1,000 ml urine).

Protein in serum was estimated by the copper sulphate method according to VAN SLYKE *et al* (1950).

Creatinine in plasma and urine was estimated according to LIEB & ZACHERL (1934). All values are the results of concordant duplicates.

Electrophoresis: free, moving boundary electrophoresis was performed with the

Tiselius-Svensson apparatus according to OLHAGEN (1945). Before the electrophoresis both urine and serum were dialyzed against the buffer (phosphat buffer, pH 7.6). The protein concentration was held about 1.2 gram per cent (one urine: 0.76 gram per cent). The reproduction (figure 6) and the estimation of the relative values was performed on the descending boundary. This electrophoresis was kindly performed by Miss Louise Edman at King Gustaf V:s Research Institute.

Filter paper electrophoresis: In most of my cases, the electrophoretic analyses of the serum and urinary protein were performed by means of filter paper electrophoresis. The method used has been worked out in a suitable manner by K.-I. BÄCKLIN, on the principles laid down by KUNKEL & TISELIUS (1951). The apparatus, made by plexi-glass, permits the parallel running of eight filter paper strips, which makes it possible to analyse serum and urine simultaneously, and in duplicate. The papers rest on a horizontal plate by means of several nibs, *i.e.* the contact with the glass is the least possible. The apparatus is during the cataphoresis covered by a lid, without contact with the papers. Paper: Whatman No. 1. Barbitallbuffer, pH 8.6 (diemal 43.6 gram, sodium hydroxide 9.6 gram and sodium acetate 31.5 gram in 5,000 ml aq. dest. The pH was adjusted to 8.6 with hydrochloric acid). Voltage 212 V. Separation time 4 hours 45 min. The strength of current was measured during one separation and was initially 9 mA, gradually increasing to 19 mA at the end of the separation. Paper sheets, 4 cm wide, were used, and serum and urine were run in duplicate. The fluid was applicated on a pencil-line across the paper with the help of a micro-pipette, and was applicated on the dry paper. The paper strips were then immediately immersed in buffer and the overload was sucked up by filterpaper before putting the strips in the apparatus.

About 10 microlitres (10 cmm) were as a rule applicated, but as far as the urines are concerned, the volume was a little less or more according to the proteinconcentration after the filtration procedure to be described below. The procedure of electrophoresis in my investigation is regarded as a qualitative analysis of the protein and the comparison of the urinary proteins have always been made with the concomitantly run serum. In that respect the *exact* protein-concentrations in the specimens to be compared are of no significance. Serum and urine were applicated without foregoing dialyzis. At the completion of the separation, the papers were dried at $+110^{\circ}\text{C}$ in a thermostate for 5—10 minutes, and then immersed for 10—15 minutes in a solution of 1 per cent bromphenol blue in absolute alcohol (with 10 per cent HgCl_2). They were then washed several times in 2 per cent acetic acid solution and finally dried at room temperature. Before photographing, the blue colour was intensified by ammonia vapor. The separation of the individual proteins was good enough to permit the cutting up of the papers according to the individual fractions in most of the cases. In some however the separation of the alfa₁-globulin was hard to decide, and there it was eluted together with the albumin. After elution of the colour in 0.01 N NaOH, the extinctions

were read in a Beckman spectrophotometer at 595 m μ . The relative concentrations of the individual proteins were calculated on the basis of their relative extinction, *i.e.* the extinction value divided by the sum of the extinctions for all the protein in the paper strip. That procedure does not account for the different binding capacity of the brom-phenol blue in the globulines. The urinary proteins are however always compared with the proteins of the serum, the electrophoretic analysis of which was always the same as that of the urine.

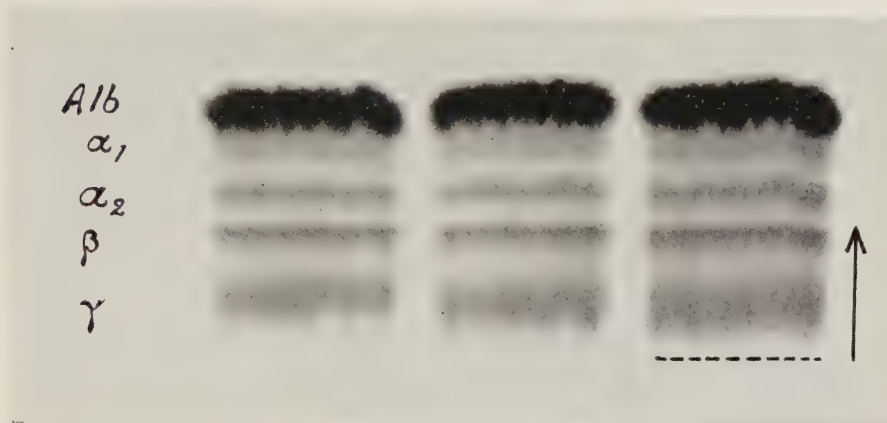


Fig. 2. Three parallelly run patterns of a serum. Dotted line indicates application zone and arrow indicates direction of migration.

Figure 2 shows three parallelly run papers with the same serum. The application line is indicated as is the moving direction towards the anode. The nomenclature is the commonly used, *i.e.* albumin, α_1 -, α_2 -, beta- and gamma-globulin. The relative concentration of each fraction in three duplicates of two serums are recorded in table I.

Concentration of the urine: to get a protein concentration in the urine of several per cent, allowing the filter paper electrophoresis to be used, the following procedure was applied: filtration of the specimen by means of protein tight ultra-filter cases (manufacturer: Membran-Filtergesellschaft Sartorius-Werke, Göttingen, Germany). The filters were attached to a glass bottle, in which a vacuum pressure was achieved by means of a motor pump. At the suction pressure of about 10 mm Hg, the atmospheric pressure induced an ultrafiltration of several millilitres of urine in a few hours. According to the original protein value the filtration was driven to a final concentration of 5–10 gram per cent. (A unitary end-concentration would have been the ideal, but for practical reasons this was not possible). The filtrates were always free from protein at the nitric acid test. Only on two occasions did precipitation of the urine make it necessary to dialyse for a few minutes against saline during the filtration procedure.

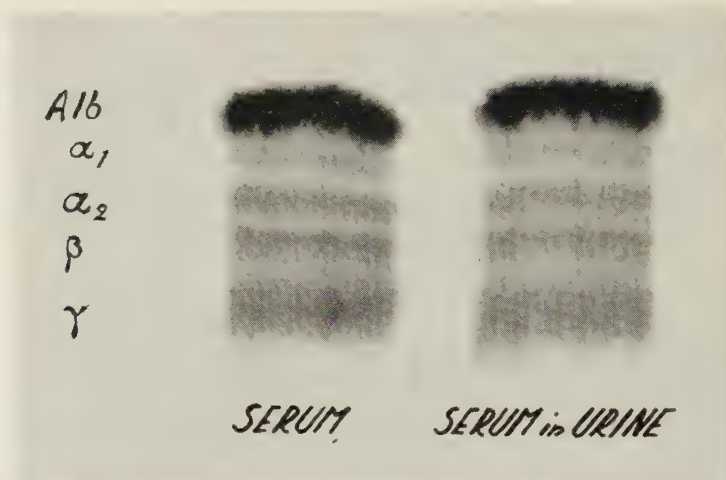


Fig. 3. Electrophoretic separation of serum and serum in urine after dilution and concentration. See text!

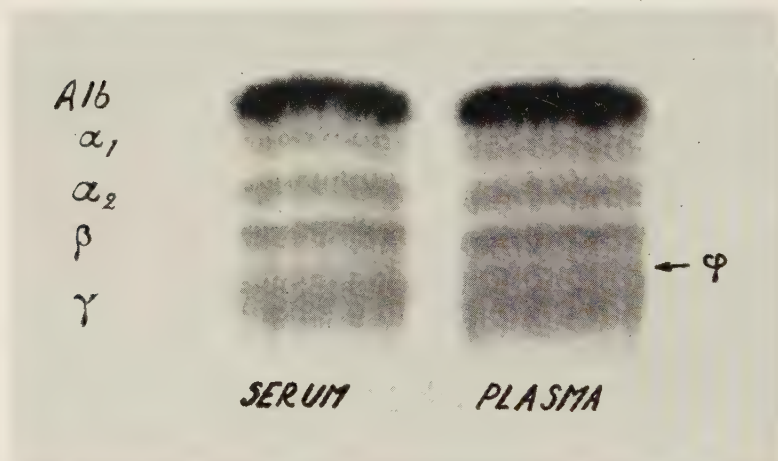


Fig. 4. Electrophoretic separation of serum and plasma. Fibrinogen migrates between γ - and β -globulin.

For a further testing of this filtration procedure, a serum was diluted with a protein-free, highly concentrated morning urine in the following manner: one part of serum in nine parts of urine. This solution, holding about 7 per mille of protein, was then concentrated by means of this ultra-filtration procedure 10 times, bringing up the final protein value to that of the original serum. Fig. 3 shows the electrophoretic analysis of the two specimens and no significant differences as to the various fractions are to be seen.

By means of this concentration method and the filter paper electrophoresis the contemporary analyses of the proteins in both serum and urine were finished in about 10—12 hours after the voiding of the urines.

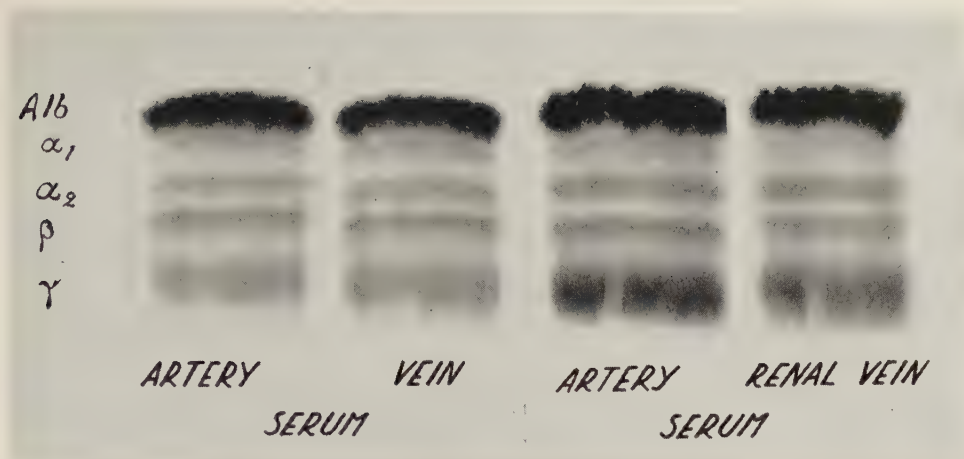


Fig. 5. Electrophoretic patterns of serum (artery and vein). To the left from a woman (V.O.C.) and to the right from a male (hydronephrosis).

The protein analysis of blood was performed on serum from a cubital vein. Significant qualitative differences between plasma and serum do not appear as far as the globulins apart from fibrinogen are concerned. Fig 4 shows the result of filter paper electrophoresis of serum and plasma (heparin-) from the same individual. Fibrinogen migrates between gamma- and betaglobulin. Nor are significant differences between venous and arterial serum (= renal artery serum) to be expected with this method. Fig. 5 shows the analysis of serum from a brachial artery and the superior caval vein (female) and a brachial artery and a renal vein (male) respectively. Table I gives the values of these various controls.

Ultracentrifuge analysis: a "Spinco" ultracentrifuge model E (manufactured by Specialized Instruments Corporation, Belmont, California, USA) was used. The analyses were performed at 59,780 RPM, which corresponds to 250,000 g. This

Table I. Filter paper electrophoresis. Relative protein values, based on relative extinction values.

Serum	Alb	α_1	α_2	β	γ
Brachial artery.....	60.8	5.4	6.8	9.4	17.7
Sup. caval vein.....	58.3	6.4	7.1	9.4	18.7
Brachial artery.....	55.4	4.5	7.0	8.0	25.0
Renal vein.....	54.9	5.2	8.3	7.7	24.0
Cubital vein.....	58.8	5.0	7.3	9.5	19.3
Serum in urine.....	58.0	6.1	8.0	9.5	18.5
Serum.....	51.8	7.1	9.2	11.3	20.6
Plasma.....	47.5	6.0	8.9	10.9	22.6
Serum (case LW) three parallelly run papers	58.7	6.3	8.1	10.2	16.7
	60.5	5.7	8.1	9.4	16.3
	58.9	6.8	8.1	10.0	16.1
Serum (case BM) three parallelly run papers	63.0		8.0	9.8	19.2
	63.0		7.8	9.4	19.8
	62.8		7.2	10.2	19.8

part of the investigation was performed at the Biochemical Department of The Medical Nobel Institute, Stockholm.

Urography: this was performed at the roentgen diagnostic department of this hospital, and in the usual routine manner, *i.e.* intravenous injection of 20 ml of a 50 per cent solution of Umbradil (Astra), and the application of an abdominal compression five minutes after the injection.

Results

The quantitative nature of the protein

In table II the urines before and after lordosis in eight cases are recorded as to their protein contents. The volume of the first specimen has not been exactly recorded in a few cases. In some cases (tables II & III) the lordosis was induced twice but on different days. In all a significant increase in the protein concentration is clear and in most of the cases this is out of proportion to the simple difference

Table II.

Case	Urine			
	Before lordosis		After lordosis	
	Volume ml	Protein $\frac{\%}{100}$	Volume ml	Protein $\frac{\%}{100}$
BL ♂.....	—	0	9	5
dR ♂.....	50	1.8	8	4.5
AS ♂.....	—	0	20	16
OL ♂.....	13	0.9	4	22
KH ♂.....	40	2.0	15	11
LW ♀.....	40	0.8	7	32
SK ♀.....	10	1.7	3	23
KL ♀.....	50	7	10	13
».....	—	+	4	27

Table III.

Case	Urine						Plasma Creatinine mg %
	Before lordosis			After lordosis			
	Volume ml	Protein ‰	Creatinine mg %	Volume ml	Protein ‰	Creatinine mg %	
BM ♂.....	167	0	234	50	8	242	1.9
AS ♂.....	—	2.5	260	—	13	416	
OL ♂.....	15	0.7	213	15	15	302	
AW ♀ a.....	50	0.7	356	3	4.5	312	1.7
» b.....	85	0	260	15	6	270	
UB ♀ a.....	33	1.0	130	26	3.0	125	
» b.....	—	0.5	281	—	5	177	1.7
LW ♀.....	—	0.3	68	—	2.3	120	
SK ♀.....	20	1.3	234	12	4.3	231	1.9
GK ♀.....	51	1.0	260	7	34	203	

in volumes. The highest values are, as in the following table, recorded in rather small volumes, however.

In the next table (No III) some urines were analysed as to their creatinine contents, before and after lordosis. No exogenous creatinine was given to these individuals. Thus the values represent the endogenous metabolite, which according to ADDIS (1948), SMITH (1951), FISHBERG (1954), and SQUIRE (1953) may be regarded as being cleared from the blood solely by filtration through glomerulus. According to ADDIS (1948) the plasma creatinine concentration can be regarded as constant at various times during the day, and may be regarded to be so in my cases during the short time these urines are secreted. (The plasma values were estimated in only four cases, with due respect to the discussions on the reliability of the estimation of the creatinine in blood.) The creatinine concentration in the specimens recorded may then be said to represent the real "concentration" of that very urine, as far as the tubular reabsorption of water is concerned. As can be seen in table III, the protein concentration is higher after lordosis, but shows no direct correlation to the concentration of this very urine specimen.

Case AW (table III line a) was able to pass the 3-ml volume 32 seconds after the standing lordotic position. Immediately afterwards she stood for another three minutes in the same position and the next specimen (13 ml) contained 13 per mille and 169 mg per cent respectively.

Case LW (table III) stood lordotic for another five-minute period after the first period (recorded in the table), and the next specimen contained 8 per mille and 73 mg per cent respectively.

The recorded values do not seem to favour a view that this protein is handled by the kidney in the same way as is the creatinine. As to the glomerular filtration during standing lordosis it is, according to earlier writers (MEDES & NEEMES 1935, RYTAND 1937, BULL 1948), very probable that it is depressed in that position. SONNE (1920), by ureteral catheterizing his patients, got anuria in most of his cases. In spite of this anuria (which was probably glomerular, as an *increased* tubular reabsorption of water in the lordotic position can scarcely be at work), he obtained some drops of fluid in one catheter, holding 26 per mille of protein.

In fact the concentration of creatinine in a urine with so high a protein content as 34 per mille in my case GK was lower than in the specimen voided before lordosis. If the glomerular filter were the place of the protein-passage, such a mechanism must involve an increased "permeability" solely for protein. That modern theory will be further discussed after the account of the qualitative nature of the protein.

The possibility of a small, but significant amount of filtered protein in the healthy human kidney, normally reabsorbed by the tubuli quantitatively, has been proposed by ADDIS (1948). If such were the case, the lordotic position could induce a cessation of that tubular mechanism, but still it must imply a glomerular filtration. That hypothesis too, will be further discussed.

The recorded values in my cases do not argue, however, against the assumption that this protein is added to the urine distally of the tubuli.

The appearance time of the lordotic protein was not measured in more than two cases, because of the difficulty in voiding on request. Only a very short appearance time, say less than a minute, would be worth relying upon, since the normal passage time from glomerulus to the bladder may lie within 2—3 minutes (SMITH). Case AW was very cooperative and voided a 3 ml specimen with significantly higher protein concentration 32 seconds after the standing lordosis. Case BD, whose urine before lordosis was free of protein, voided 5 ml proteinholding urine 1 min 45 sec. after a lordotic standing. But as the standing position in both cases was held for five minutes, the time values cannot be said to argue significantly against the glomerulus as the "source" of this protein. And of course these values lose all significance, if a collecting tubular change were the explanation of this protein.

The sediments

Post-lordotic sediments have been examined in all cases.

As all urines were voided freely, the qualitative examination is mainly related to erythrocytes and casts.

Erythrocytes: in no case there was any hematuria. The examination revealed in five cases one or two red cells in several view fields, but such values have occasionally been observed in the routine sediments, and cannot be looked upon as pathological. Case KL, who before lordosis revealed clearly pathological values (5—10 red cells per view field in several examinations) did not display any increase of it in lordotic specimens with 13 or 27 per mille of protein. In the other cases only occasionally red cells were observed in the whole sediment.

Casts: Case KH showed in the lordotic urine several hyaline casts per view field, which is an increase above the normal, and a few granulated cylinders were observed in the whole sediment. This makes a renal disease suspicious, but the absence of hematuria argues against glomerular lesion. A diffuse renal disease is not probable, in view of the other normal findings (see case report) but some pathological nephrons cannot be excluded. Slightly increased amounts of hyaline cylinders were observed in case AS and dR, that is a few casts were observed per view field. In the other cases only a few hyaline casts within the normal range were observed. Urines with high protein values, such as 22 and 34 promille did not exhibit a single cast. The lordotic urine of case LW in table II was unfortunately not examined as to the sediment.

Spermatozoons have never appeared in the lordotic urines of the males.

In most of the urines, a significant increase in epithels and white corpuscles were observed after lordosis. The epithels have always been the big squamous cells typical of the lower urinary passages, but nothing else can of course be said as to the origin of these cells than that they do not derive from the nephron.

The absence of erythrocytes argues against any severe lesion of the glomerulus in the standing lordotic position.

The absence of hyaline casts in specimens of high protein concentration argues against the view that this protein derives from the glomerulus. However, the formation of casts within the kidney still involves some unknown factors, but the following is worth reviewing. OLIVER (1944) has given an account on this problem. Based on his great morphologic knowledge of the nephron and experimental findings he believes that the formation of casts takes place in the distal convolution and the collecting tubulus, and not proximal of this level. He considers that the phenomenon includes stability-changes in a system, comprising four factors: electrolytes and urea, the protein (concentration and nature), the H-ion concentration and a fourth factor (non-dialyzable and heat-resistant) which he designates the "X-body".

As to the lordotic urines in my series, the protein concentration may be regarded as high in several specimens. As will be shown later the protein includes globulins as well as albumin. The creatinine concentration in the lordotic urines is still high. Nothing can of course be said about the pH of these urines during their tubular passage. I, myself, have made no investigations in the intricate problem of the so called "acetic acid body" in my urines. It has been known since a long time, however, that the urine of benign proteinurics is especially rich in the "acetic acid body", *i.e.* a substance, which characteristically facilitates the coagulation of the urinary protein, giving a coagulation already by acetic acid in the cold. POLLITZER (1912) demonstrated a heavy concentration of that substance in the urines of healthy young people with benign proteinuria contrary to those of elderly people, and stressed the concordance of that substance with MÖRNER'S (1895) protein-precipitating substance, including chondroitin-sulfuric acid. MÖRNER demonstrated the presence of chondroitin-sulfuric acid in the cortex and the medulla of the kidney. However, OLIVER'S "X-body" corresponds in several important respects with the same substance. Thus, as a matter of fact, the urinary protein, and according to the literature also the protein-precipitating factor seem to be in abundance in benign proteinuria, and still the absence of casts in such urines are the rule. That speaks in favour of a view that the urinary protein in benign proteinuria has not passed the nephron from the glomerulus.

The qualitative nature of the protein

Electrophoretic and ultracentrifugal analyses

Free, moving boundary electrophoresis was performed in three cases. The relative concentration values must of course be taken with the precautions necessary in the judgment of every indirect method of estimations of the various fractions of protein. This is the more important here, as different methods have been used

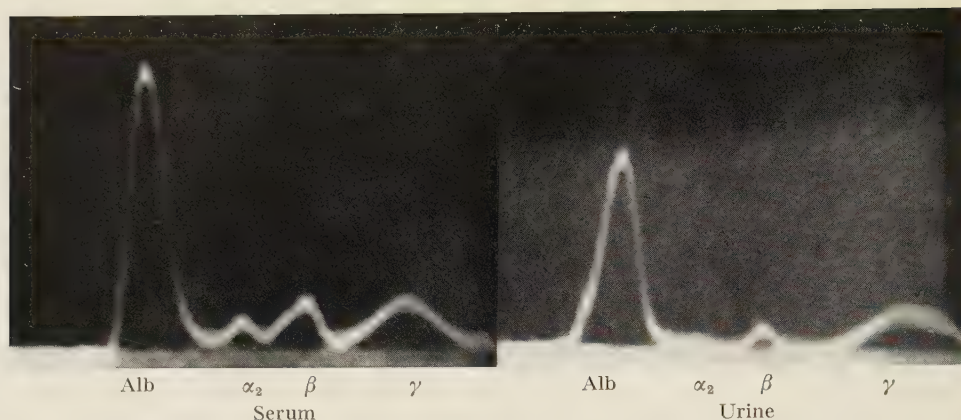


Fig. 6. Electrophoretic diagrams (free moving boundary method) from serum and urine. Case BM.

The following values from the three cases were recorded.

Case	Protein					
	total—%	alb.	α_1	α_2	β	γ
BM ♂ serum.....	8.4	56.7	2.8	6.6	12.4	21.5
urine.....	0.8	53.4	3.3	6.9	11.0	25.4
KH ♂ serum.....	8.4	58.2	3.2	7.4	14.0	17.2
urine.....	1.1	62.6	2.7	5.3	6.7	22.7
GK ♀ serum.....	7.0	53.3	3.7	9.4	14.9	18.7
urine.....	3.4	60.4		5.7	7.5	26.4

for the total and the relative values. But as far as serum is concerned, the total protein values are within normal limits. Fig. 6 shows the diagrams for serum and urine of case BM.

Thus it seems clear that all fractions, included in the serum, have been refound in the urine. With due regard to the possible over-estimation of the urinary globulins (urinary mucoids may be included in the gamma-values for example) there seems to be a grossly normal relation between the various protein-fractions of urine and serum.

Ultracentrifugal analysis

(this part of the work was performed in collaboration with Mr Anders Ehrenberg at the Biochemical Department of the Medical Nobel Institute, Stockholm. Head: Professor Hugo Theorell).

As the electrophoretical procedure says nothing about the molecular weights of the various proteins, the determination of their sedimentation constants by means of the ultracentrifuge method has been performed on some post-lordotic specimens.

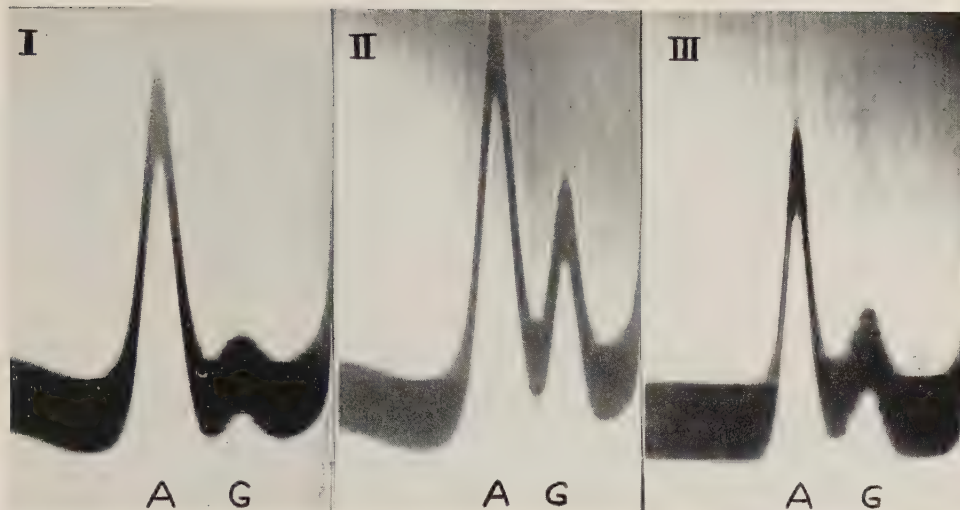


Fig. 7. Ultracentrifuge diagrams. I = urine case OL. II = same urine with added γ -globulin. III = solution of albumin (2 %) and γ -globulin (2 %) in one per cent saline. "A" denotes albumin fraction, "G" denotes globulin fraction. See text!

The sedimentation constant may be used for an approximate estimation of the molecular weight of a protein, an important characteristic in the discussion of the proteinuria mechanism.

Figure 7 I shows the diagram of urine (case OL). No II is the diagram of the same urine, to which was added human gamma-globulin* (0.1 ml of a 12 per cent solution to 1 ml of urine). No III is the diagram of an *in vitro* mixture of human albumin* (2 per cent) and gamma-globulin (2 per cent). The urinary globulin part in diagram I has been identified as having closely the same sedimentation velocity as the gammaglobulin in diagram II and III.

The observed differences could totally be ascribed to normal concentration effects. Moreover in diagram II the peak of the original urinary globulin and that of the added gamma-globulin seems to coincide perfectly. In control experiments it was shown that the two commercial proteins alone had the same sedimentation constants that have been previously determined (PEDERSEN 1945).

Diagram No III gives an example of the behaviour of a mixture of albumin and globulin in the ultracentrifuge. The areas of the two peaks do not correspond to the known proportions of the two proteins ($1/1$ in diagram III). The significance of this phenomenon has been earlier reviewed by McFARLANE (1935). The globulin part in a mixture is always underestimated when it is calculated from the area in a sedimentation diagram. Hence all data of the globulin percentage given in table IV must be regarded as minimal values. For practical reasons the ultracentrifugal analysis could not be accomplished on fresh specimens either but only on

* Manufacturer: AB KABI, Stockholm.

Table IV. Ultracentrifugal analyses. Urine specimens after lordosis. Total protein values in urines represent the actual concentration after voiding.

Case	Total protein %	Albumin S ₂₀	Globulin S ₂₀	Albumin rel %	Globulin rel %
BM ♂ serum	8.4	3.72	5.46	87	13
urine	0.8	3.94	~ 6.4	93	7
AS ♂ urine	1.5	3.86	5.62	90	10
OL ♂ urine ³	2.2	3.32	4.88	90	10
KH ♂ urine	1.1	3.66	5.37	90	10
AW ♀ serum	7.5	3.98	5.03	85	15
urine	0.6	3.80	6.47	92	8
urine ¹	1.3	3.53	5.16	90	10
UB ♀ urine	0.3	3.92	5.80	87	13
LW ♀ urine	0.8	4.08	6.28	91	9
BM ♂ serum in urine ²	0.8	3.80	5.50	88	12
OL ♂ urine + γ -glob. ³		2.98	4.36	69	31
γ -glob. (0.5 per cent)		—	6.56	—	100
γ -glob. (2.0 " ")		—	5.80	—	100
Albumin 2 per cent					
+ γ -glob. 2 per cent ³		3.74	4.86	75	25
		S ₂₀ ^o	S ₂₀ ^o		
Albumin (human)...		4.67	—		
γ -glob. (human)....		—	7.12		
(according to PEDERSEN 1945) ...					

¹ = fresh specimen.
² = one part serum in nine parts urine (proteinfree, voided before lordosis).
³ = see fig. 7.

refrigerated urines, and in some cases the resolution by warming up the specimen again in room temperature was probably not complete. This source of error may especially influence the globulines.

In table IV the calculated sedimentation constants S_{20} (corrected for temperature and known salts) have also been included. All urines investigated showed a globulin part similar to that of diagram I in figure 7. All samples of urine or serum were investigated at total protein concentrations of 0.7—1.2 gram per cent. The sera were diluted with a $1/15$ molar phosphate buffer of pH 6.9 containing 1 per cent sodium chloride.

JAHNKE & SCHOLTAN (1953) in a recent study by means of ultracentrifugal analyses of urinary proteins assumed that a proteolytic splitting of the globulins takes place in the kidney in chronic renal diseases (nephrosclerosis, chronic glomerulo-nephritis and diabetic nephropathia) as a discrepancy was found as to the globulin part between urine and serum. But they did not pay due attention to the errors mentioned above in calculating the proportion of globulin by means of the ultracentrifugal diagram.

Filter paper electrophoresis

This method had the advantage of simultaneously analysing the serum and urinary proteins. In some cases urine before and after lordosis were included. All

Table V. Filter paper electrophoresis of proteins.
(urine 1 = before, 2 = after lordosis)

CASE	S E R U M						U R I N E						
	Total %	Alb	α_1	α_2	β	γ	No	Total %	Alb	α_1	α_2	β	γ
BL ♂...	8	57.6	6.5	8.1	10.2	17.6	2	5	67.5	5.6	4.3	9.7	12.6
LV ♂...	7.5	65.3	5.1	5.6	9.5	14.5	1	+	73.7	5.0	3.0	7.7	10.6
							2	+	68.8	6.5	4.6	8.9	11.3
dR ♂...	8.5	60.5	6.5	7.8	9.1	16.0	1	1.8	68.5	6.4	4.1	8.7	12.3
							2	4.5	67.3	6.0	4.2	8.7	13.9
BD ♂...	8	63.9	5.4	6.7	8.9	15.1	2	+	72.0	4.5	4.4	8.9	10.4
		66.2	4.7	6.4	7.8	15.0 ¹							
KE ♂...	8	57.3	5.6	7.3	10.8	19.0	2	22	68.8	5.3	3.3	9.1	13.6
OL ♂...	7	66.8		9.4	8.2	15.7	2	22	67.0		5.8	8.9	18.4
AW ♀...	7.5	58.2	6.7	8.4	10.3	16.5	2	13	65.5	6.8	5.4	9.7	12.6
UB ♀...	8	65.6	5.5	5.8	9.5	13.7	2	3	72.5	4.7	3.2	9.1	10.5
LW ♀...	7	61.3	5.2	8.2	8.9	16.4	1	0.8	73.5	5.1	3.7	8.3	9.3
							2	32	76.3	4.4	3.3	7.4	8.6
SK ♀...	7.5	59.8	6.1	6.6	10.5	16.9	1	1.7	73.7	5.0	3.1	8.0	10.3
							2	23	70.4	5.1	3.3	10.1	11.1
GF ♀...	7.5	58.4	5.5	8.7	10.2	17.3	2	+	55.0	6.3	7.7	13.7	17.3
GA ♀...	7.5	58.7	6.4	10.1	8.9	15.9	1	+	69.7	5.4	4.8	8.0	12.1
							2	+	61.3	6.5	5.8	11.5	14.9
KL ♀...	8	58.8	6.3	9.3	9.8	15.8	1	7	68.5	5.9	5.5	8.7	11.3
							2	13	61.4	6.7	5.8	11.2	14.8

¹ = serum in urine (see text).

specimens were run in duplicate. Thirteen individuals were investigated in this manner. Three cases have been reported on as to free electrophoresis. The handling of one specimen (case AS) was unfortunately unsuccessful as I applied another method for concentration of the urine, which caused a precipitation of proteins not resolvable.

The values are recorded in table V. The cutting of the paper, elution of the colour, and the reading of the extinction values include some sources of error, however. And as my discussion concerns the qualitative nature of the urinary proteins, representative reproductions are given. (In some of the pictures a pale zone is visible in the gamma-fraction, due to some optical error in the photographing procedure.)

Figure 8 gives the result of cases BL and KE. Also by means of this method all fractions included in the serum are recovered in the urine. However, the relative proportions are diverging. There seems to be included some more albumin than in the corresponding serum, and further there appears to be relatively more beta-globulin in these urines than α_2 globulin, contrary to what appears in the serum picture. This type of diagram has been found in every urine analysis according to this filter paper electrophoresis technique. It is quite obvious visually, but it is reflected in the table, too, in most of the specimens after reading the values in the Beckman spectrophotometer. A slight predominance of albumin in most

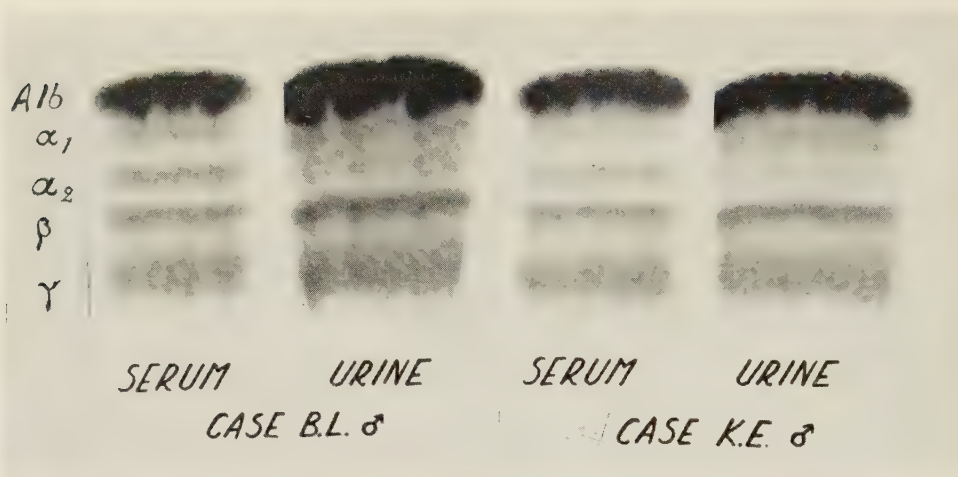


Fig. 8. Electrophoretic separation of serum and urine (urine after lordosis).

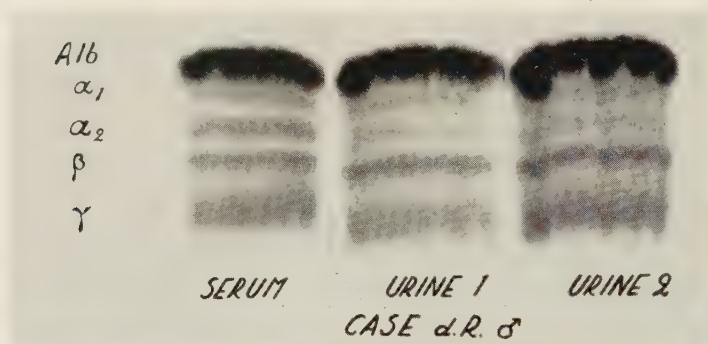


Fig. 9. Electrophoretic separation of serum and urine before (1) and after (2) lordosis.

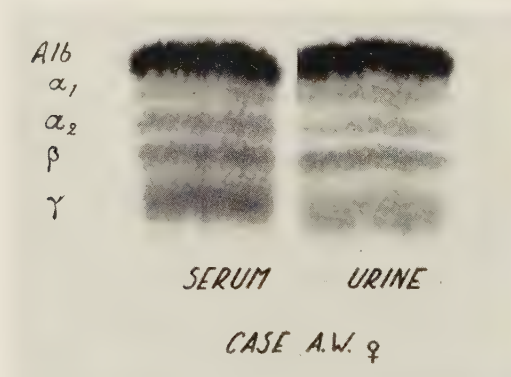


Fig. 10. Electrophoretic separation of serum and urine (after lordosis).

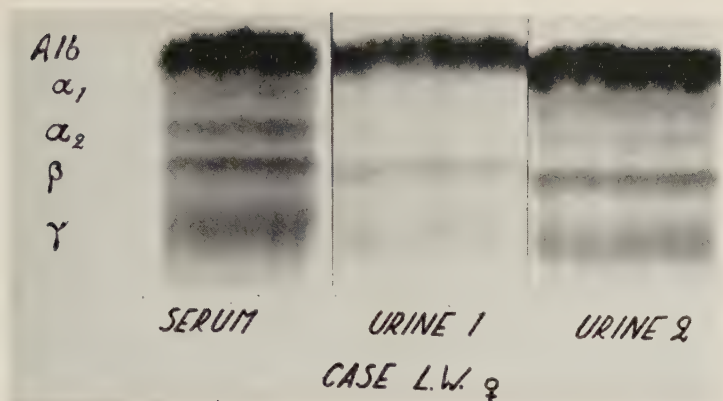


Fig. 11. Electrophoretic separation of serum and urine before (1) and after (2) lordosis.

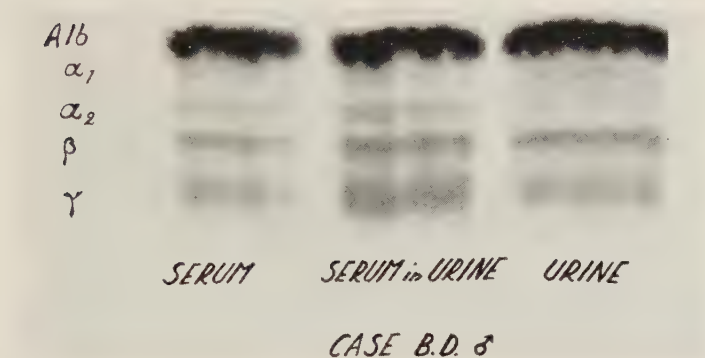


Fig. 12. Electrophoretic separation of serum, serum in urine (after dilution and concentration, see text) and post-lordotic urine.

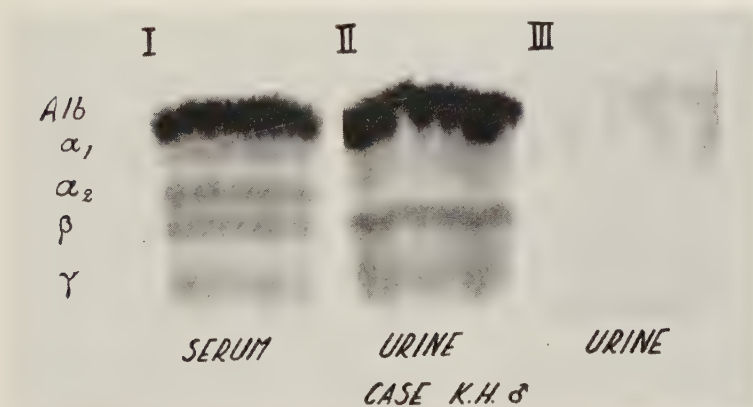


Fig. 13. Electrophoretic separation of serum and urine. I and II: protein colouration. III: lipid colouration (Sudan black).

urines in comparison with serum is evident, and in most of the urines the relative gamma-globulin values are somewhat lower. In the urines the relative values of beta-globulin are of the same range as those of serum, and there seems to be a higher quotient β/α_2 in the urines as compared with serum. No evident difference can be found, however, between urines with a high and urines with a low protein value (before the concentration of the sample) from the same individual. Nor is there any significant qualitative difference in specimens as to their actual protein concentration during the electrophoretic procedure. This is evident in figures 9 & 11. The cutting of the α_1 -zone was difficult in some papers, but its value does not seem to influence my further discussion.

Figure 9 shows the result of case dR before and after lordosis. Figure 10 shows the analysis of case AW (specimen after the second lordotic position, see page 29).

Figure 11 gives the result of case LW, where the protein values in the two urines were 0.8 and 32 per mille respectively.

There seems to be no difference as to the qualitative relations of the proteins before and after lordosis. Nor is any significant difference to be found between males and females.

Case BD voided before lordosis a protein-free urine. To this was added serum in relation 1: 10, and the filtration procedure was applied to this mixture and driven to one tenth, giving a final concentration as the original serum. The parallelly run papers including urine after lordosis are shown in fig. 12 where the serum proteins are shown to be unchanged as far as the electrophoretic separation is concerned.

A closer investigation of the urinary beta-globulin was not made in this connection. However, a lipid coloration method (SWAHN 1953) was applied in three cases (dR, KH and GA) following SWAHN's technique as to the drying of the paper etc. The serum picture was in that respect normal. In one case only does the concentration of the Sudan black permit a photographic reproduction (figure 13) and a concentration of presumable Sudan coloured lipoids are to be found in the albumin-region. No Sudan is to be found in the beta-line. (Only the values from the free electrophoretic protein-analysis of case KH have been included here). There are presumably no lipo-proteins of the beta-type, normally found in serum, in these urines. Nothing can be said with certainty of the lipoids in the albumin region. According to SWAHN (1953) they may be regarded as α -lipoproteins. The method seems to prove that lipoids are included in these urines. A proteinfree urine was for control concentrated next to nil, and no lipoids were identified, following the method above.

The electrophoretic analysis according to the filter paper method has proved my above assumption that the urinary protein after lordosis does not differ from a qualitative point of view from the protein "excreted" in the usual daily conditions. By means of this method all protein fractions included in serum were also identified in the benign proteinuria. The urinary protein "spectrum", seemingly quite typical for the benign proteinuria, diverges in some respects from the serum picture.

Urography

All cases were subjected to urography by the intravenous method. In case BD this procedure disclosed a malformed kidney on the right side, with clubbed calyces and rather thin parenchyma. The left kidney was somewhat enlarged (presumably compensatory hypertrophy) but otherwise quite normal. In all the other cases the urograms were normal as far as topography, size, configuration, dye-excretion, the out-lines of the pelvis, the calyces, the ureters and the bladder are concerned. No tumor, calculus or inflammatory changes were revealed.

General discussion

The following discussion will mainly deal with the problem of the renal mechanism of the passage of protein from circulating plasma to the urine. An account of the modern views on that mechanism may be given as a basis for the discussion of my own results.

However, the following opinions still involve, as far as the various forms of renal diseases are concerned, some matters of dispute.

I. A prerenal mechanism

i.e. proteinuria is explained in a humoral way.

The glomerular filter is regarded as normally permeable for proteins according to their molecular weights, letting smaller molecules through. The limit as to the molecular weight is thought to lie in the order of a weight slightly lower than that of serum albumin. The investigations by BAYLISS, KERRIDGE & RUSSEL (1933) regarding the passage of egg-albumin and Bence-Jones protein found an experimental basis for such a prerenal view. As to human renal diseases, WUHRMANN & WUNDERLY (1950) maintain the hypothesis of some "dysproteinämie" of the plasma as an explanation of the proteinuria in nephrosis and even in benign proteinuria, *i.e.* a prerenal, humoral mechanism. Their view is however not shared by other modern writers. The hypothetical prerenal mechanism laid down by ADDIS (1945, 1948) in some cases of an apparently benign proteinuria has been mentioned above. Further studies in that line did not prove the assumption. As a matter of fact, one of his cases revealed milky effusions in the abdominal and the pleural cavities. The patient died and "postmortem suggested obstruction of lacteal duct" was added to the case report (ADDIS 1948) without further commentaries.

Nothing in my investigation can support the conception of a prerenal proteinuria as the explanation of the benign urinary protein.

II. A renal mechanism

i.e. proteinuria is the result of some disorder in the nephron.

First possibility: The normal glomerulus is believed to filter plasma protein in small, but significant amounts which is reabsorbed by the tubulus. In view of the modern conception of the largeness of the daily glomerular filtrate (SMITH 1951) such a filtering of protein would quantitatively suffice to explain even high grade proteinuria, if the tubular reabsorption is depressed in some way or other. This opinion was especially emphasised by ADDIS (1948). The experiments made by WEARN & RICHARDS (1924), EKEHORN (1931) and WALKER, BOTT, OLIVER & MACDOWELL (1941) on animals do not support the view of protein normally filtered through the glomerulus, however. BING, in a recent paper (BING & HARBOE 1952) still is of the opinion, too, that the normal glomerular filtrate is protein-free. As far as the healthy human kidney is concerned, no data are available to support normal protein filtering, either. Modern investigations on the nephrotic syndrome by BARNETT, FORMAN & LAUSON (1952) and CHINARD, LAUSON, EDER, GREIF & HILLER (1954) do not support the view, that the nephrotic proteinuria can solely be explained by a decreased tubular reabsorption of normally filtered protein.

My own values cannot possibly be explained by such a mechanism, either. From the quantitative point of view my values cannot, however, argue against such a hypothetical mechanism. Considering the concentration index (*i.e.* $\frac{\text{urine creatinine mg \%}}{\text{plasma creatinine mg \%}}$) as laid down by BING (1936) as a measure of the final concentration of the voided urine from the glomerular capsule, and further assuming no tubular re-absorption of the supposed filtered protein, the value for case GK (table III) after lordosis does not exceed the hypothetical values as proposed by ADDIS among others (a normally filtered amount in the magnitude of 30 mg % in the capsular urine). But from the qualitative point of view (*i.e.* albumin and globulins as proved by the electrophoretic and ultracentrifugal analyses) no sound discussion seems to be possible concerning any re-absorptive function of the tubular cells. Especially not when the agreeing patterns for the specimens before and after lordosis are concerned. Above all, the qualitative picture of the urinary protein can hardly be assumed to be the result of normally filtered protein in the glomerulus. If the benign proteinuria were due to protein filtered through the glomerulus, an increased permeability for protein (and solely for protein) must be assumed to be at work, *i.e.*:

Second possibility: Proteinuria is explained by an increased permeability for protein in the glomerulus filter.

Practically all modern writers are of the opinion that this is the important change in the glomerulus that explains the urinary proteins, as well in the various forms of renal diseases, as apparently in the benign proteinuria, too. This assumption

of a specifically increased permeability in the filter for protein, but not for electrolytes and water, was thoroughly reviewed by EKEHORN (1931), and the problem is still a matter of dispute. Up to the present, the hypothesis lacks any proof, however, that the protein in the glomerular capsule really conforms qualitatively to the protein found in the urine voided from the same kidney.

Also in view of this opinion, so widely embraced, the analysis of the protein in my individuals, as to the proportions of the various globulins and their sedimentation constants, cannot be said to fit into any filtering mechanism of the glomerulus of healthy kidneys. And no evidences can be given for a view that the glomerular filters of these kidneys suffer any functional disturbance during the normal daily activity of the individual.

My assumption that the urinary protein before and after lordosis does not differ qualitatively has been proved. The assumption that the renal mechanism proper, *i.e.* the place for the passage of protein from plasma to urine, is the same before and during lordosis may be regarded as sound. What really happens to the glomerulus during lordosis can hardly be determined. However, as to the filtering of water and electrolytes per unit time, *i.e.* the clearance, the literature has given evidences for the decreased function in that respect (MEDES & NEEMES 1935, RYTAND 1937, BULL 1948). That is to say, the lordosis decreases the filtration but it certainly enhances the protein output. In that very important respect the lordotic proteinuria differs from the assumptions made by BING (1936) (as far as various renal diseases are concerned), from the proteinuria in experimental constriction by the renal artery (OVERBECK 1863), and from the proteinuria in the experiments made by BAYLISS, KERRIDGE & RUSSEL (1933), where the filtration and diuresis continuously decreased in the transplanted kidney, without, however, a concomitant increase in the proteinuria.

Thus, there seems to be no support for the view of the glomerular filtering of the urinary protein in benign proteinuria, especially not during lordosis.

As far as the lipoids are concerned, the opinion diverges in the literature also as to the renal handling of them. BING (1936) and FISHBERG (1954) for instance believe that they are filtered *via* glomerulus. OLIVER (1944), CAVELTI (1948, 1949) and FREY (1951) on the other hand believe, that they do not pass the glomerular filter (as far as the nephrotic syndrome is concerned at least) but are derived from the tubular epithelium, being the products of metabolic processes there.

I remind of the above discussion on the cast formation in the kidney. The qualitative nature of the urinary proteins should favour the formation of casts even in the following respect: BLACKMAN, GOODWIN & BUELL (1941) have given evidences for the assumption that the presence of globulins in the tubular urine (besides albumin) favours the precipitation of the protein. By applying more modern methods (electrophoresis) for the protein analysis BLACKMAN & DAVIS (1943) further emphasise the importance of gamma-globulin in that respect.

To sum up: the idea of glomerular filtration of the proteins in my cases is not supported on account of the following facts:

No correlation between filtered creatinine and protein; the qualitative nature of the urinary protein regarding electrophoresis and ultracentrifugal analysis; the presence of presumable lipoproteins in these urines; absence of casts in specimens of high protein concentrations.

Third possibility: Proteinuria is explained by tubular excretion of protein.

This view is denied by ADDIS (1948), SMITH (1951), BARNETT, FORMAN & LAUSON (1952), SQUIRE (1953) and by CHINARD, LAUSON, EDER, GREIF & HILLER (1954), especially with regard to the important renal disorder, designed as "the nephrotic syndrome". This is the more difficult to understand, as the histological changes in the nephrotic kidney certainly primarily affect the tubulus.

KAISERLING (1942), FREY (1949, 1951) and JAHNKE & SCHOLTAN (1953) believe that tubular excretion of protein is at work in the nephrotic syndrome.

The part of the nephron to which the tubular interference in the proteinuria mechanism is related is the proximal tubulus. This is clear from the data by OLIVER (1939, 1944), SMETANA (1947) and RATHER (1948) as far as the tubular reabsorption of protein is under discussion, and from the data by KAISERLING (1940, 1942) as far as the possible tubular excretion is concerned.

Despite the disagreement in the literature as to the important question of the possible excretory mechanism for protein in the tubulus there can be little support for a view that the urinary protein in my cases is due to such a mechanism. Nor can any anatomical support be found for a protein excretion located in the collecting tubulus.

III. A post-renal mechanism

i.e. the protein derives from below the nephron.

Such a mechanism is commonly thought to be involved only in the case of inflammatory disorders of the pelvis or the lower urinary passages (apart from chyluria).

No supports for the mechanisms reviewed under I and II have come true. Several facts speaking in favour of a post-renal admixture of protein have been emphasized in part one of this book, and as a matter of fact no other mechanism can logically explain the results of the various analyses in my series, *i.e.* the quantity and the quality of the urinary protein, the absence of casts in concentrated specimens and the creatinine values. The discrepancy found between the creatinine and the protein values can easily be explained by the assumption that the urine specimen after lordosis in reality includes urine from the dead space in the urinary passages, filtered and concentrated in the nephron *before* the lordosis.

With regard to this post-renal view, my values cannot differ between various anatomic locations in the urinary passages. There is however little anatomic support for a protein admixture from the urethra or the bladder, or even the



Fig. 14. Intravenous pyetogram case LW. Pyelo-sinuous refluxes from several calyces right kidney. Picture taken 24 minutes after injection, showing contrast in the sinus renalis.

ureter. As suggested in the first part there are some observations pointing to the renal pelvis, especially then the fornix of the calyx.

By the urographic investigations of my cases, there were in two individuals revealed refluxes of the pyelo-lymphatic type, *i.e.* via the fornix calyces. In case LW there appeared bilateral refluxes as soon as 10 minutes after the abdominal compression was applied, and to an unusual extent.

The figure 14 taken 24 minutes after the injection, shows contrast lying just outside the pelvic wall in the sinus renalis. The lordosis study (table II) on this

individual was performed about five weeks after the urography. The proteins in her urines before and after lordosis do not differ from a qualitative point of view from those of the other cases, but the value of 32 per mille in the lordotic specimen may be regarded as unusually high. Case KL, who had been urographied before the lordosis study, too, gave a specimen of 27 per mille, still with the same analytic values. Case GK, where the lordosis was induced before the urography, gave a specimen holding 34 per mille. Urography did not give rise to any refluxes in her case, nor in any other cases.

The lordotic position was induced before urography in the following cases: BL, LV, BD, BM, KE, OL, KH, UB, GF and GK. Thus, the urography per se by no means have induced any changes in the pelvis of those kidneys, which could explain the proteinuria.

The fornix of the calyx in the renal pelvis has earlier been emphasised as being an anatomic space, with but scarcely understood functions in normal physiologic conditions, but some well-known clinical and experimental disorders have been proved to be connected with that part of the pelvic mucosa.

In my opinion, and referring to the review of the renal lymphatic system, the fornix obviously offers a place within the kidney where the intimate connection between proteinholding fluid (*i.e.* the lymph) and the urinary passages could explain the admixture of protein more easily than any hypothesis concerning an ultrafiltering, but in normal healthy conditions certainly protein-retaining membrane as the glomerular filter.

Nothing can be said about the very passage of the protein in such an assumption, except that it can scarcely be looked upon as being brought about by any "rupture" of this epithelium.

Nothing in my analyses contradicts the view of lymph as being the source of the urinary protein. Especially not when the view held by DRINKER (1942) concerning the quotient albumin/globulin in lymph, is regarded, *i.e.* this quotient is slightly higher in lymph than in plasma. The absence of fibrinogen can be explained in the same way, *i.e.* it is in the healthy state only sparingly at hand in the lymph (von WITTICH 1881, DRINKER). Above all: the electrophoretical picture of the urinary protein as brought forth by means of the filter paper method, in some respects apparently typical of all the included individuals, could most easily be explained, if evidences for such a picture could be obtained from renal lymph.

A normal human kidney could scarcely be obtained for such a prooftaking. By courtesy of my colleagues at the urologic department of this hospital I had the opportunity of examining some extirpated kidney to that purpose. I did not succeed in taking any sample from a renal lymph vessel without the admixture of blood. Several attempts were unsuccessful so far as a strong haemolysis occurred in the specimens obtained, too, and the haemoglobin happens to migrate in the buffer employed by the electrophoretic procedure exactly like beta-globulin. Nor could the analysis be relied upon as an evidence, if being performed on fluid from grossly

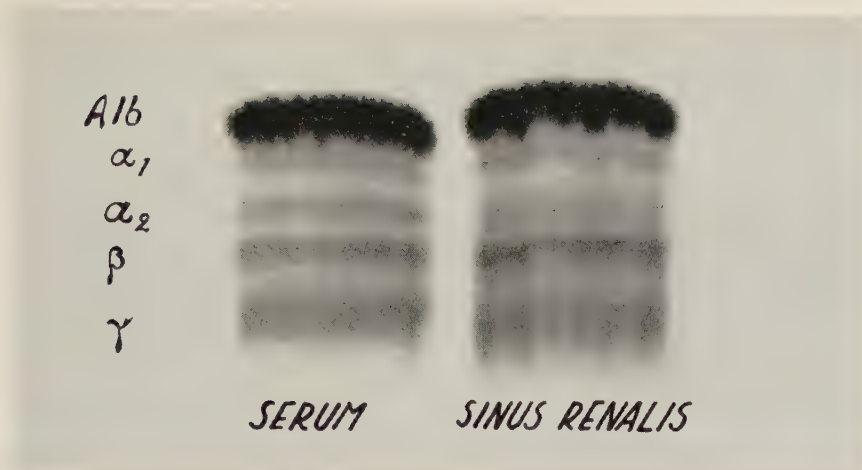


Fig. 15. Electrophoretic patterns of serum and sinus-renal fluid; (case: Hydronephrosis, male).

abnormal kidneys. A case of hydronephrosis without general infection symptoms and without past or present symptoms of inflammation in the kidney (no proteinuria, no bacterial growth on culture, normal NPN and erythro-sedimentation rate) and without evident pyelo-nephritic changes in the later histological examination, was however successful in that respect. But also in that case, no pure lymph was obtainable. The fluid was taken, however, immediately after the extirpation directly from the sinus renalis. Any fluid taken there may be a mixture of blood and lymph. If the protein diagram differs from serum, taken in direct connection with the extirpation, it must, however, presumably include another proteinholding fluid too, *i.e.* lymph. The specimen was left to settle down in a test tube, was then centrifugated, and the supernatant fluid and the serum, exactly 10 cmm of each, were applied on the paper to run simultaneously.

Figure 15 gives the result of that procedure. It is evident that the diagram from sinus renalis differs slightly from that of the serum, but in the same respect as the diagrams of the urinary proteins in my series. Even in this specimen haemoglobin was included, but only detectable by means of a handspectroscope. The concentration, estimated according to DRABKIN & AUSTIN (1935), was in the magnitude of 0.05 gram per cent, which is out of order to interfere significantly.

Another sample from the sinus renalis was successful, too; in that case however, the kidney was suffering a pyelo-nephritis on the basis of ureteral calculus. Traces of haemoglobin were included macroscopically, but sufficient fluid for a quantitative determination was not obtained. Regardless of that, the electrophoretic values from the sinus renalis also differ from the serum taken during the operation. The following values were obtained:

	<i>Alb</i>	α_1	α_2	β	γ
Hydronephrosis: serum (tot. prot. 6 %).. (male)	56.9	6.6	7.2	11.3	17.9
sinus renalis.....	58,5	6.7	5.8	11.4	17.6
Pyelonephritis: serum..... (female)		58.9	12.4	11.2	17.6
sinus renalis.....		67.5	6.1	13.1	13.3

Two analyses of that kind can of course not stand any statistical procedure. The more important objection will also be that any comparison between such protein-containing fluid as that taken in sinus renalis and the urine should concern the sinus and the urine from the same individual.

My discussion, as well as my own investigation procedures, have mainly stressed the mechanism proper for the proteinuria, *i.e.* regardless of any probable alterations in the blood circulation.

The widely accepted view of the importance of a disturbance of the venous return from the kidney, so often emphasized in the literature during the last half century, may simply be explained by the facts laid down by DRINKER, as far as the general lymphatic system is concerned, and by KAISERLING, as far as the renal lymphatics are concerned: a venous stasis increases the flow of *lymph* from the organ in question.

The logically presumptive influence on the lymphatic flow by hydrostatic changes has been pointed out. It also seems logical that a lordotic spine should at first hand influence a flow running parallelly with the columna, such as the lymph on its way from the abdomen to the upper body half, but scarcely be able to "compress" perpendicularly running vessels, such as the renal vein.

It need not be re-emphasized that other alterations in the flow of lymph, a fluid system that in today's medicine seems to have been rather neglected, may influence several normal and pathological functions of the kidney.

Conclusion

The mechanism of the benign proteinuria has been studied by means of own investigations, and the very mechanism of the passage of protein into the urine has been especially considered. The quantitative and qualitative nature of the urinary protein, the lacking correlation to the functions of the glomerulus (such as the filtration of creatinine) and the absence of casts are emphasized as evidences for a post-renal mechanism, *i.e.* the protein is admixed to the urine distally of the nephron. The fornix of the calyx of the renal pelvis is pointed out as the possible place of such a discharge of protein from the renal lymphatic system.

Summary

- 1) After a short discussion of the nomenclature, a review is given of the literature on the benign proteinuria, with special regard to the varying theories as to its mechanism.
- 2) The disagreement in the literature, especially concerning the supposed connections with the arterial and the venous circulation is emphasized and the widely embraced conception of the glomerular filtration of the urinary protein is criticized.
- 3) The possible relation between the benign proteinuria and the renal lymphatic system, earlier proposed by QUINCKE, is accounted for.
- 4) Evidences based on own investigations are given for a new comprehension of the benign proteinuria, *viz.* that this proteinuria is a post-renal proteinuria, where the protein is admixed to the urine distally of the tubulus.
- 5) Evidences from the literature on the renal lymphatic system, especially considering the sinus renalis Henle, and from own investigations, are given for the assumption that this urinary protein derives from the renal lymphatic system, and that the passage of lymph takes place *via* the fornix of the calyx in the renal pelvis.

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